

## EFFICACY OF BIOCONTROL ANTAGONISTS AGAINST ROOT KNOT

### NEMATODE, *MELOIDOGYNE INCOGNITA* INFECTING TOMATO

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#### ABSTRACT

Experiments were conducted in net house to assess the biocontrol potential of liquid formulation of *Pseudomonas fluorescens* and *Bacillus subtilis* on the multiplication of *Meloidogyne incognita* and growth of tomato plant cv. Pusa ruby. Among the different treatments evaluated, drenching the pot soil with either of the bioagents @ 10ml/liter at transplanting and again at 30days after transplanting recorded the maximum growth and biomass of tomato plant with decreased root knot nematode multiplication, galls and egg masses. Drenching the soil @ 10ml/liter at transplanting alone was equally effective in significantly enhancing the plant growth and reducing the nematode infection. This treatment with *P. fluorescens* resulted 15.9% increase in shoot height, 16.4% in shoot dry weight and 18.4% in root dry weight. Host infection in terms of number galls and egg masses per plant was decreased by 33.6 and 32.6% respectively. However, *B. subtilis* was found better in reducing nematode multiplication and improving plant growth than *P. fluorescens*. Drenching with *B. subtilis* @ 10ml/liter at transplanting resulted 19.2, 58.1 and 43.1% improvement in shoot height, dry weight of shoot and root, respectively, whereas 62.2 and 62.3% reduction were recorded in number of galls & egg masses respectively, as compared to untreated check.

**KEYWORDS:** *Pseudomonas Fluorescens* and *Bacillus Subtilis* on the Multiplication of *Meloidogyne Incognita* and Growth of Tomato Plant cv. Pusa Ruby

#### INTRODUCTION

Tomato (*Solanum lycopersicum* Mill; Family - Solanaceae) is one of the largest grown vegetable crop in the world. India ranks second in the area as well as in production of tomato. In India, area under tomato is 865 thousand ha with production of 16826.0 thousand tons and productivity of 19.5 tons/ha. Andhra Pradesh is the largest producer of tomato in India contributing about 35.22% to the total production whereas Odisha occupies third place in production contributing only about 8.13% of tomato in the country. Tomato is rich source of vitamins A, C, potassium, and minerals. Tomatoes are used in the preparation of soup, salad, pickles, ketchup, puree, and sauces, also consumed as a vegetable in many other ways. Lycopene is the bright red carotenoid pigment and phytochemical found in tomatoes. Lycopene as an antioxidant prevents cancer of breast, colon, rectum, stomach, lungs, ovaries, pancreas and prostate. It is also used to prevent diabetes, cardiovascular diseases, cataracts, and asthma.

Root-knot nematodes (*Meloidogyne* spp.) cause serious damage on a wide range of crops, especially on vegetables such as tomato, potato, eggplants, okra etc. in tropical and subtropical agriculture (Sikora and Fernandez, 2005;

Anamika et al., 2011). Second stage juveniles ( $J_2$ ) of *Meloidogyne* spp. can infect plant roots at the early growing stage, and the invading populations develop as the root systems mature. They migrate to the vascular cylinder, induce severe root galling and ravage the utilization efficiency of water and nutrients even though adequate levels of these are available in the soil. Consequently the nematode infection of plants leads to stunted growth, wilting, and poor fruit yield. The most characteristic symptoms of the disease occur below ground. Roots develop multiple profuse galls; these galls often fuse to cause extensive swelling and distortion of the root system (fig. 1).

Four major species, namely *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla* and *Meloidogyne arenaria* have been reported to infect tomatoes, but *M. incognita* has been found dominant and a major limiting factor in the tomato crop production causing an average yield loss of 24-38% (Netscher and Sikora, 1990) and 28-68% (Pakeerathan et al., 2009). In India 39.7 and 27.2% reduction in tomato yield was reported by Reddy, 1985 and Jain et al., 2007, respectively due to *M. incognita*.

## REVIEW OF LITERATURE

Among various biocontrol agents, Fluorescent pseudomonad's, equipped with multiple mechanisms for biocontrol of phytopathogens including plant parasitic nematodes and plant growth promotion, are being used widely as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase, and can solubilize phosphorous (Yeole & Dube, 1997; Dileep et al, 1998; Senthilkumar et al., 2008).

Sirohi et al., (2000) indicated the toxicity of *Pseudomonas fluorescens* filtrate to juvenile of *Meloidogyne incognita*. Mortality was about 80% or higher at S/50 and higher concentration of *Pseudomonas*. The active ingredient for toxicity got destroyed on boiling the culture filtrate, indicating possibility of its proteinaceous nature. However no significant difference was observed in mortality for unheated and heated culture filtrate of *Pseudomonas* suggestive of its non proteinaceous nature.

Jothi et al. (2003) evaluated the efficacy of commercially formulated *Pseudomonas fluorescens* against root-knot nematode, *Meloidogyne incognita*, race 3 infesting tomato. *P. fluorescens*-treated plants gave the maximum yield (64.3%) and minimum *M. incognita* soil population (56%).

## MATERIALS AND METHODS

Experiments were conducted under pot culture condition in the net house of O.U.A.T., Bhubaneswar. Various materials and methods used in the present investigation have been categorized and detailed below.

### Preparation of Soil and Pots

Well pulverized soil, free from plant debris and pebbles, was collected from the University central farm. The soil was mixed thoroughly with sand and FYM in the ratio of 2:1:1 which was autoclaved at 15 lb pressure/ sq inch for 20 minutes. Earthen pots of 15 cm diameter were cleaned and surface sterilized with 4% formaldehyde solution. Pots were air dried and then filled with aerated autoclaved soil.

### Maintenance and Culturing of Nematode

The experimental pure culture of root-knot nematode, *Meloidogyne incognita* was originally obtained from a single egg mass progeny maintained and multiplied on brinjal seedlings in 30 cm diameter pots containing autoclaved soil.

The population of nematode was sub-cultured periodically at 50-60 days intervals by inoculating new transplants with infective second stage juvenile suspension of *Meloidogyne incognita* obtained from old culture pots. Other intercultural operations were attended to as and when necessary.

### Isolation of Egg Masses and Juveniles of *M. Incognita*

Galled roots of brinjal plants were collected from the culture pots. The roots were washed free from soil under a tap with gentle stream of water. After partial air drying egg masses are picked up with the help of tweezers and needles and kept over the wire gauze - tissue paper assembly rested on the petridish containing clean tap water just touching the bottom surface of the wire gauze. Petridishes were also covered to avoid evaporation loss. Freshly hatched second stage juveniles ( $J_2$ ) were collected in beakers at 24 h intervals and fresh water was added to the petridish at each change. Collection of juveniles was continued for 7 to 8 days and used subsequently for experimental purpose.

## RESULTS

Two pot culture experiments were conducted in the net house of the Department of Nematology during 2013 – 14 with the following objectives.

- To study the efficacy of *Pseudomonas fluorescens* (liquid formulation) as soil drench against root knot nematode, *Meloidogyne incognita* infecting tomato.
- To study the efficacy of *Bacillus subtilis* (liquid formulation) as soil drench against root knot nematode, *Meloidogyne incognita* infecting tomato.

The data on different plant growth parameters and nematode multiplication were statistically analyzed, compared with untreated as well as chemical check and presented in this chapter.

### Bio-Management of Root Knot Nematode, *Meloidogyne Incognita* Infecting Tomato by Using *Pseudomonas Fluorescens* Liquid Formulation as Soil Drench

A pot culture experiment was conducted for the management of root knot nematode, *Meloidogyne incognita* by using *Pseudomonas fluorescens* liquid formulation as soil drench treatment in tomato. For this purpose, two dilutions (5ml & 10ml per liter of water) of commercial liquid formulation of *Pseudomonas fluorescens* were chosen. One month old tomato seedlings (var. Pusa ruby) from nursery pots were carefully uprooted washed thoroughly and were transplanted (one in each pot) in 15 cm diameter earthen pots containing 1kg autoclaved soil. One thousand freshly hatched second stage juveniles of *Meloidogyne incognita* were inoculated to each pot. On the next day, the pot soil was drenched with aqueous solution of *P. fluorescens* of either 5ml or 10ml per liter or with different combinations. One untreated inoculated check was also maintained. The experiment was terminated after two months of nematode inoculation. Observations were recorded on different plant growth parameters and nematode multiplications. Photographs pertaining to relative shoot and root length as well as root galling are presented in Fig. 2 & 3.

It would be seen from data (Table-1; Figure. 4.A) that all the treatments were able to increase the shoot height ranging from 7.89 to 17.23% as compared to inoculated check. Maximum increase (17.23%) in shoot height was observed with *Pseudomonas fluorescens* soil drench @ 10ml/liter at transplanting and again at 30 days after transplanting ( $T_7$ ). This was followed by the treatment with *P. fluorescens* soil drench @ 10ml/liter at transplanting only ( $T_3$ ) in which shoot height was increased to the tune of 15.89% as compared to untreated check plant. The shoot height was increased to 15.73% in

the treatment where *P. fluorescens* was used at both transplanting (@10ml/liter) and 30 days after transplanting (@5ml/liter), respectively ( $T_6$ ). These three treatments ( $T_3$ ,  $T_6$  &  $T_7$ ) were statistically at par with each other. However, other treatments of *Pseudomonas* were not significantly effective as compared to untreated check.

The data on fresh weight of shoot (Table-1; Fig. 4.B) indicated that various treatments could increase the shoot weight to different degrees (6.77 to 36.83%) over check. Maximum increase in the fresh shoot weight to the tune of 36.83% was recorded in the treatments when the pot soil was drenched with *P. fluorescens* at transplanting and also at 30 days after transplanting @10ml/liter ( $T_7$ ). Treatment with *P. fluorescens* soil drench @ 10ml/liter at transplanting ( $T_3$ ) as well as  $T_3$ + *P. fluorescens* soil drench @ 5ml/liter at 30 days after transplanting ( $T_6$ ) registered 23.06 and 28.04% significant increase respectively in shoot weight over the check. Analysis of the data showed that the above treatments ( $T_3$ ,  $T_6$  &  $T_7$ ) were statistically at par.

With regard to shoot dry weight (Table-1; Figure 4.C); the data also indicated an increase in weight as compared to inoculated check. The percentage of increase ranged from 5.22 to 19.97%. Maximum increase in the dry shoot weight (19.97%) was recorded, when the pot soil was drenched with *P. fluorescens* liquid formulation @ 10ml/liter at transplanting and also at 30 days after transplanting ( $T_7$ ). The treatment with *P. fluorescens* @ 10ml/liter at transplanting ( $T_3$ ) recorded 16.41% increase in dry shoot weight where as *P. fluorescens* soil drench @ 10ml/liter at transplanting + *P. fluorescens* @ 5ml/liter at 30 days after transplanting ( $T_6$ ) resulted in 18.58% increase. Statistical analysis of data showed that there were no significant differences between the above three treatments.

**Table: 1. Effect of *Pseudomonas fluorescens* Liquid Formulation as Soil Drench on Shoot Growth of Tomato Infected with *M. incognita* (Average of 4 Replications)**

Treatments	Shoot Height (cm)	% Decrease	Shoot Fresh Weight (g)	% Decrease	Shoot Dry Weight (g)	% Decrease
$T_1$ = Untreated check	18.75	-	27.75	-	7.86	-
$T_2$ = Pf 5ml/liter at transplanting	20.23	7.89	29.63	6.77	8.27	5.22
$T_3$ = Pf 10ml/liter at transplanting	21.73	15.89	34.15	23.06	9.15	16.41
$T_4$ = $T_2$ + Pf 5ml/liter at 30DAT	20.35	8.53	30.38	9.48	8.33	5.98
$T_5$ = $T_2$ + Pf 10ml/liter at 30DAT	20.48	9.23	30.88	11.28	8.58	9.16
$T_6$ = $T_3$ + Pf 5ml/liter at 30DAT	21.70	15.73	35.53	28.04	9.32	18.58
$T_7$ = $T_3$ + Pf 10ml/liter at 30DAT	21.98	17.23	36.83	36.83	9.43	19.97
SEM (0.05)	0.63	-	2.49	-	0.45	-
CD (0.05)	1.86	-	7.33	-	1.31	-

Pf= *Pseudomonas fluorescens*; DAT= Days after transplanting

The root length increased over inoculated check in all the treated plants (Table-2; Figure 4.D). It ranged from 3.74 to 16.26%, the maximum being at  $T_7$  (drenching with *P. fluorescens* liquid formulation @ 10ml/liter at both transplanting and 30 days after transplanting). This was followed by the treatment with *P. fluorescens* @ 10ml/liter at transplanting + 5ml/liter at 30 days after transplanting ( $T_6$ ), where the root length was increased by 14.78%. Drenching the pot soil with *P.*

fluorescens @ 10ml/liter at transplanting could register 14.58% increase in root length. However, the statistical analysis of data revealed non-significant differences among the treatments.

Data on root fresh weight (Table-2; Figure 4.E) showed that the weight increased in all the treatment in comparison to inoculated check. The percentage of increase in the root system ranged from 3.40 to 25.30%. The treatment with *P. fluorescens* @ 10ml/liter at transplanting and also at 30 days after transplanting ( $T_7$ ) registered maximum percentage of increase in root mass followed by 22.53% increase in the treatment with *P. fluorescens* @ 10ml/liter at transplanting + 5ml/liter at 30 days after transplanting ( $T_6$ ). The treatment with *P. fluorescens* @ 10ml/liter at transplanting only ( $T_3$ ) could register 20.55% increase. Statistical analysis of data revealed that the above treatments ( $T_7$ ,  $T_6$  &  $T_3$ ) were at par in increasing the fresh root weight of tomato plants

With regard to root dry weight (Table-2; Fig. 4.F) all the treatments recorded increase in weight over check. Such increase was minimum (7.77%) in soil drenching with *P. fluorescens* @ 5ml/liter at transplanting ( $T_2$ ). Treatment with *P. fluorescens* @ 10ml/liter at transplanting and also at 30 days after transplanting ( $T_7$ ) registered maximum increase over check plants (20.49%). The treatment with *P. fluorescens* @ 10ml/liter at transplanting + 5ml/liter at 30 days after transplanting ( $T_6$ ) registered an increase up to 19.43% over the check plant. This was followed by the treatment with *P. fluorescens* @ 10ml/liter at transplanting ( $T_3$ ), which recorded 18.37% increase. The statistical analysis of data showed that soil drenching with *P. fluorescens* @ 10ml/liter at transplanting and @ 5 or 10ml/liter at 30 days after transplanting ( $T_3$ ,  $T_6$  &  $T_7$ ) were at par.

**Table: 2. Effect of *Pseudomonas fluorescens* liquid Formulation as Soil Drench on Root Growth of Tomato Infected with *M. Incognita* (Average of 4 replications)**

Treatments	Root length (cm)	% decrease	Root fresh weight (g)	% decrease	Root dry weight (g)	% decrease
$T_1$ = Untreated check	10.15	-	12.65	-	2.83	-
$T_2$ = Pf 5ml/liter at transplanting	10.53	3.74	13.08	3.40	3.05	7.77
$T_3$ = Pf 10ml/liter at transplanting	11.63	14.58	15.25	20.55	3.35	18.37
$T_4$ = $T_2$ + Pf 5ml/liter at 30DAT	10.85	6.90	13.55	7.11	3.08	8.83
$T_5$ = $T_2$ + Pf 10ml/liter at 30DAT	11.18	10.15	13.75	8.70	3.13	10.60
$T_6$ = $T_3$ + Pf 5ml/liter at 30DAT	11.65	14.78	15.50	22.53	3.38	19.43
$T_7$ = $T_3$ + Pf 10ml/liter at 30DAT	11.80	16.26	15.85	25.30	3.41	20.49
<b>SEM (0.05)</b>	<b>NS</b>	<b>-</b>	<b>0.88</b>	<b>-</b>	<b>0.12</b>	<b>-</b>
<b>CD (0.05)</b>		<b>-</b>	<b>2.58</b>	<b>-</b>	<b>0.35</b>	<b>-</b>

Pf= *Pseudomonas fluorescens*;

DAT= Days after transplanting

Perusal of data (Table-3; Figure 5.A) on the number of galls per plant revealed a reduction over the untreated check in response to different soil drench treatments. The minimum reduction (8.49%) in number of galls was obtained when pot soil was drenched with liquid *P. fluorescens* @ 5ml/liter at transplanting ( $T_2$ ). Maximum reduction (38.15%) in number of galls was obtained in the treatment where *P. fluorescens* was used @ 10ml/liter drenching at transplanting and

also at 30 days after transplanting ( $T_7$ ). It was followed by 35.22% decrease in the treatment with *P. fluorescens* @ 10ml/liter at transplanting + 5ml/liter at 30 days after transplanting ( $T_6$ ) and 33.56% decrease with *P. fluorescens* @ 10ml/liter at transplanting ( $T_3$ ). Statistical analysis of data did not reveal any significant differences between these three treatments ( $T_7$ ,  $T_6$  &  $T_3$ ).

Data on number of egg masses/plant (Table-3; Figure 5.B) indicated that all the treatments were able to reduce the egg masses in the root system. Maximum decrease in the number of egg mass (35.66%) was obtained in the treatment where soil drenching was done with *P. fluorescens* @ 10ml/liter at transplanting and also at 30 days after transplanting ( $T_7$ ). This was followed by 34.24% decrease in  $T_6$ , where pot soil was treated with *P. fluorescens* @ 10ml/liter at transplanting + 5ml/liter at 30 days after transplanting ( $T_6$ ). *P. fluorescens* @ 10ml/liter at transplanting ( $T_3$ ) caused 32.56% reduction in egg mass formation in comparison to untreated. Statistical analysis of data revealed no significant differences between the above three treatments ( $T_7$ ,  $T_6$  &  $T_3$ ).

With regard to the final nematode population of  $J_2$  in 200cc soil, the data (Table-4; Fig. 5.C) revealed that the reduction of population was ranged from 11.25 to 25.91%. The maximum significant reduction (25.91%) was obtained in ( $T_7$ ) where soil drenching was done with *P. fluorescens* @ 10ml/liter at transplanting and also at 30 days after transplanting with the same dose. This was followed by 24.78% decrease with *P. fluorescens* @ 10ml/liter at transplanting + also at 30 days after transplanting @ 5ml/liter ( $T_6$ ) and 24.37% decrease with *P. fluorescens* @ 10ml/liter at transplanting alone ( $T_3$ ). However, there were no significant differences between these three treatments ( $T_7$ ,  $T_6$  &  $T_3$ ).

## DISCUSSIONS

Tomato (*Solanum lycopersicum* Mill.) is one of the most important commercial and widely grown vegetable crops in both tropics and sub- tropics. It has been estimated that plant parasitic nematodes cause global losses to crop plants to the tune of \$118b annual losses to world crops annually (Atkinson et al., 2012). Though the average annual yield loss for major crops due to root-knot nematodes is 12.3% (Sasser and Freckman, 1987), yet it is as high as 24-38% as reported by Netscher and Sikora (1990) and 27.2% for tomato (Jain et al., 2007). Four major species, namely *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla* and *Meloidogyne arenaria* have been reported to infect tomatoes, but *M. incognita* has been found dominant and a major limiting factor in the tomato crop production in major production regions. Root knot nematodes are sedentary obligate endoparasitic nematodes on a wide range of crops. *Meloidogyne* spp. can infect plant roots at the early growing stage, and the invading populations develop as the root systems mature. Second stage juveniles ( $J_2$ ) penetrate the roots and migrate to the vascular cylinder, induce severe root galling and ravage the utilization efficiency of water and nutrients and greatly affect photosynthetic products. Consequently the nematode infection of plants leads to foliage symptoms including stunted growth, wilting, and poor fruit yield.

## SUMMARY AND CONCLUSIONS

Root-knot nematodes, *Meloidogyne incognita*, recognized as among the most economically important and complex group of plant parasitic nematodes, cause damage and high yield losses on most cultivated plants particularly vegetable crops throughout the world especially in developing countries. Tomato (*Solanum lycopersicum* Mill.) is one of the most important commercial and widely grown vegetable crops in both tropics and sub- tropics, which is often severely attacked by root-knot nematode, *Meloidogyne incognita*, a predominant and widely prevalent species inflicting serious loss in tomato. In recent years, continuing environmental problems associated with the use of nematicides have introduced a

sense of urgency into the search for alternative methods of nematode management. Bacteria are the most abundant microorganisms in the root zone and their presence can significantly modify the rhizosphere environment and affect directly or indirectly the nematode or the host-parasite interrelationship. Of these rhizobacteria, *Pseudomonas fluorescens* and *Bacillus subtilis* referred to as plant growth promoting rhizobacteria (PGPR) are two widely used antagonists against *M. incognita*.

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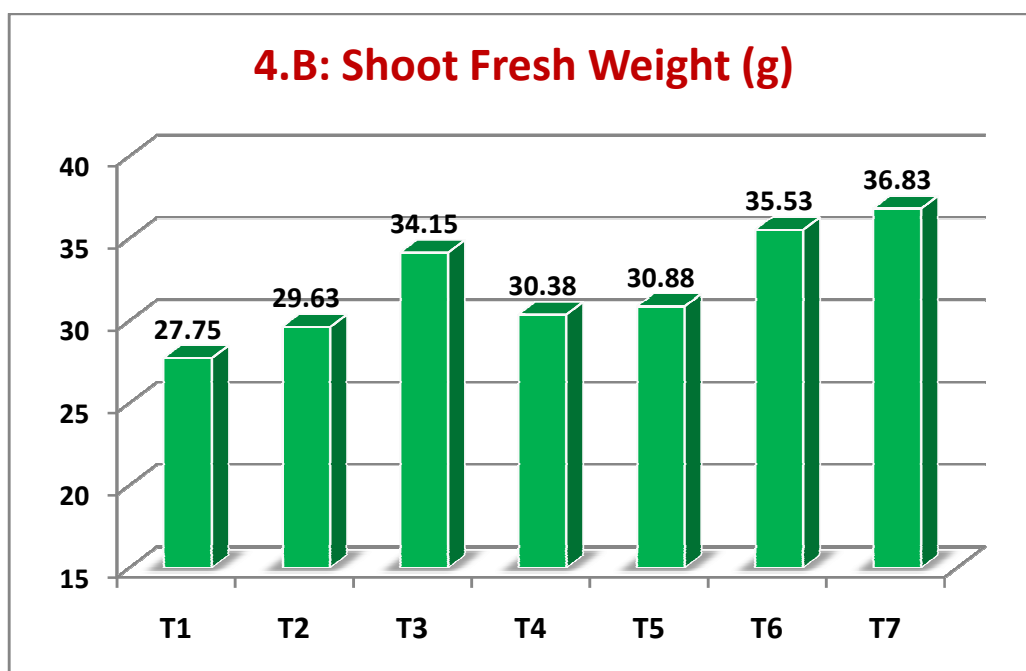
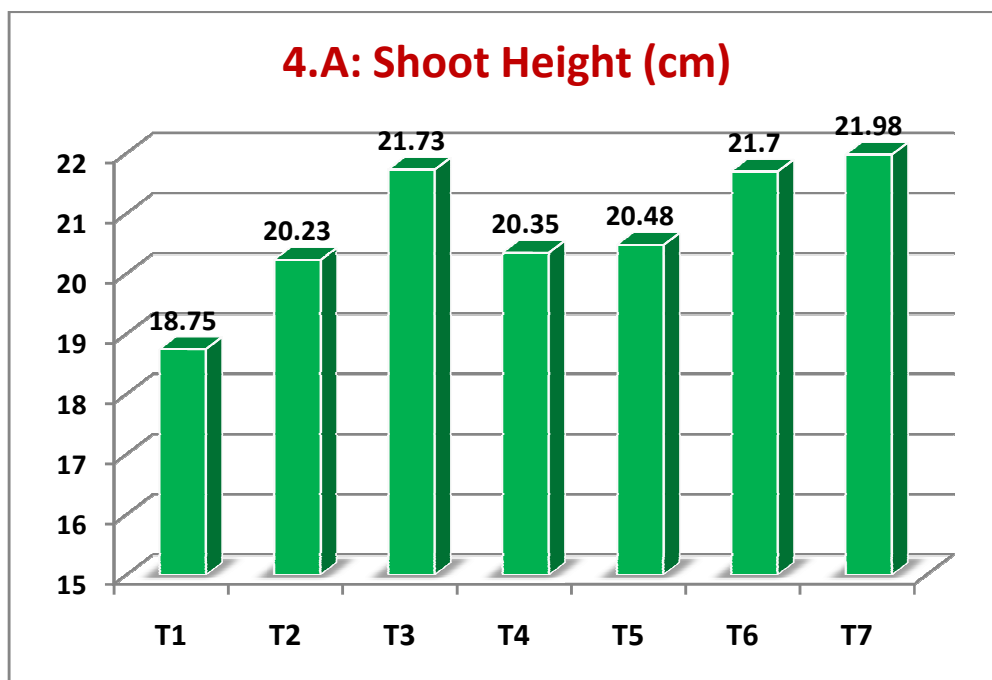


Figure- 4: Effect of *Pseudomonas Fluorescens* Liquid Formulation as Soil Drench on Plant Growth Parameters (Tomato Var-Pusa ruby)

T<sub>1</sub>= Untreated check

T<sub>2</sub>= Pf @ 5ml / liter at transplanting

T<sub>3</sub>= Pf @ 10ml / liter at transplanting

T<sub>4</sub>= T<sub>2</sub>+ Pf @ 5ml / liter at 30 DAT

$T_5 = T_2 + Pf$  @ 10ml / liter at 30 DAT

$T_6 = T_3 + Pf$  @ 5ml / liter at 30 DAT

$T_7 = T_3 + Pf$  @ 10ml / liter at 30 DAT